VOLUME I

SCIENTIFIC LITERATURE REVIEW ON THE SAFETY OF MONOSODIUM GLUTAMATE

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SCIENTIFIC LITERATURE REVIEW

ON

THE SAFETY OF MONOSODIUM GLUTAMATE

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INTERNATIONAL GLUTAMATE
TECHNICAL COMMITTEE

PREFACE

There are available in May 1974, review documents concerning MSG, such as,

1) FAO Nutrition Meetings Report Series No. 48A, 1970
2) NAS/NRC "Safety & Suitability of MSG for use in baby foods", 1970
3) Scientific Reports on the Safety of MSG (Ajinomoto Co., Inc.), 1970
4) MSG, A Review of Efficacy & Safety Vol. I-IV (IMC Corp. & Ac'cent Int'l Inc.), 1969-73
5) Scientific Literature Review (Tracor Jitco Co.)Feb.1974

From the toxicological point of view and the safety evaluation of food additives, the necessity of more up-dating and complete summary of all the available data was strongly felt.

After scrutiny of all the available papers accumulated up to the present, exact summaries from original papers were abstracted as objectively as possible.

Application, Production and Analysis are intentionally excluded in order to concentrate on toxicology.

Some new data which have remained unpublished were supplemented.

This summary is hoped to provide all the up-dated toxicological data necessary for the evaluation of safety-in-use of MSG.

CONTENTS

Summary

SUMMARY

All the available chemical and biological data concerning the safety of glutamates are reviewed in detail and their main essential points are summarized as follows:

Glutamic acid occurs in combined form as a common constituent of animal and plant protein as well as in free form in various tissues of living organisms. Monosodium L-glutamate, the sodium salt of L-glutamic acid is used to enhance the palatability of foods. In the Orient, it is used to impart a characteristic taste to food.

The biochemical role and metabolism of glutamate in mammals is well established. The available pathways for metabolism of exogenous glutamate is also known. Blood levels of glutamate are not changed substantially, both in animal and human subjects after moderate oral doses of glutamate (0.2 g/kg). A massive dose is required to produce a significant increase in blood glutamate levels in most individuals.

There are a number of reports on the extensive pharmacological studies of exogeneously administered glutamate. Iontophoretical applications of L-glutamate are found to cause excitation of many neurones in the central nervous system, and seizures induced by large doses of MSG were suggested to be related to the effect of glutamate. The effect of MSG on behavior has been reported in experimental animals.

Acute toxicity, subacute and chronic toxicity, reproduction, teratology, mutagenicity and carcinogenicity are described. Dietary levels of approximately 10% or more of MSG or L-glutamic acid for an extended period of time may induce some effects such as reduction in body weight or organ weight change in some animal species. No obvious adverse effect of MSG or L-glutamic acid on reproduction or teratogenic findings have been noted in mice, rats and rabbits. As measured by tumor incidence in mice and rats through their life span, there is no evidence that MSG is carcinogenic. A dominate lethel assay in mice and a host mediated assay in rats showed no mutagenic activity of MSG.

Single or successive injections of MSG can cause retinal lesions in neonatal mice and rats, and adults rabbits. Oral administration of MSG apparently does not produce any retinal lesion in these animals or in squirrel monkeys fed MSG.

Brain lesions can be induced in infant mice (0-12 days) given more than 0.5 g/kg of MSG, and in adult mice only with subcutaneous administration of MSG at 5 g/kg or more. There is no evidence that the brain lesion was induced by dietary feeding. The character of the brain lesion was noted to be necrosis of the neuronal or non-neuronal cells in the arcuate nucleus of the hypothalamus. Monosodium L-aspartate, L-cysteine and L-glutamic acid are reported to induce the lesion in mice. Sodium chloride also caused lesions which

were different in the site and the nature from those produced by MSG. In rats, subcutaneous administration of 2 g/kg or more, and oral administration of 4 g/kg MSG, induced the acute brain lesion as that of mice. However, negative results were also reported. In dogs and monkeys, all the papers (except two reports on monkeys by one author) failed to demonstrate the brain lesions.

Massive ingestion of MSG in susceptible persons has been suggested to cause a certain kind of syndrome, the so-called "CRS". Similar symptons, however, were also observed in volunteers of a control group. All of the symptoms characteristic of CRS are subjective and transient. Several experiments by the double blind method have shown that there are no significant differences in the incidence of symptons between control and treated groups with 3 to 4.4 g doses of MSG in a general unselected population.

Introduction

INTRODUCTION

Several seasoners with flavor-enchancing properties have been used among Asian countries since old times and have played as an important role as salt or sugar in increasing eating pleasure. The substances, all of which were derived from natural sources, have been chosen as such through experience. Some of them were found to be more effective when used together with another. Recently with the development of science and technology, the useful components of these enhancers of natural origin have been isolated, and the industrial mass-production of flavor enhancers has become possible. They have rapidly come into wide use both in processed foods and family kitchens on account of their preservability, economical aspects and handiness.

Glutamic acid is one of the most common of the amino acids and is a constituent of practically all proteins. It is not an essential amino acid, but has important metabolic roles such as that of an amino-doner in the synthesis of other amino acids in living organisms. Monosodium L-glutamate (MSG)* is a sodium salt of L-glutamic acid, and has been used as a flavor enhancer or a seasoner for processing foods and home cooking in many countries for a long time.

(*MSG: Monosodium L-glutamate used as a flavor enchancer)

Numerous scientific studies on the chemical and biological properties of glutamate have been reported. In 1968, adverse effects of glutamate on experimental animals or human beings were suggested by several reports in the U.S.A. Thus, consumers and regulatory authorities in some countries, together with the United Nations, showed renewed interest in the safety of glutamate.

This report is a review of scientific literature concerning chemical, biochemical, toxicological and physiological properties of glutamates. The literature cited here includes data which are available through April, 1974 and are necessary for the safety evaluation of glutamates.

1. Brief historical background

For centuries, Japanese people have used many traditional food seasoners such as seaweed, soy sauce, dried bonito, etc. In 1908, Ikeda isolated the essential component of taste from a seaweed of the Laminaria sp. He found that seaweed extracts contained the glutamate which has a characteristic taste, different from sweet, sour, salty and bitter taste (Ikeda, 1909). Glutamic acid was first isolated from hydrolysate of wheat gluten by Ritthausen in 1866, and was synthesized by Wolff in 1890.

The industrial production of MSG began in Japan in 1908 (Ikeda, 1908). MSG had been produced by a hydrolytic process, in which wheat gluten or defatted soybean protein is used as a raw material (Ikeda, 1908).

In 1956, Kinoshita et al, reported a new fermentation process for producing glutamate, in which carbohydrates such as glucose or sucrose were used as a main raw material (Kinoshita et al., 1961). MSG is now produced by two kinds of processes: mostly by a fermentation process, and in smaller amounts, by a protein hydrolytic method. A snythetic process had been developed since 1962, but at present, is not used (Oeda, 1963).

2. Organoleptic properties

The taste of MSG is reported to be characteristic, different from other basic tastes, sweetness, sourness, saltiness and bitterness. Ikeda proposed that this palatable taste be classified as another basic taste, "Umami" in Japanese, or "glutamic taste" (Ikeda, 1909). Cairncross reported that glutamate had a sweet saline taste accompanied by some astringency (Cairncross, 1948). Solms investigated the nature and taste intensities of many amino acids, in which L-glutamate proved to have a complex taste difficult to evaluate in pure state, described as "unique, glutamic" (Solms, 1969). Galvin described that glutamate in distilled water at a concentration of 0.05 to 1.0% was just slightly sweet and slightly salty. Most foods are eaten with appreciable amounts of salt being present. Glutamate with salt produced a taste that was mildly sweet and pleasantly salty, but having an additional effect of an apparent high flavor intensity. The most similar taste was that of a salted chicken broth, although the broth was lacking in this high intensity of flavor (Galvin, 1948).

Crocker reported that MSG appeared to be entirely without odor when pure, and its taste in solution had all four components; sweetness, sourness, saltiness and bitterness (Crocker, 1948). The taste characteristic of MSG was studied organoleptically and it was found that the taste of MSG consisted of taste like glutamate 71% saltiness 13.3%, sweetness 9.8%, sourness 3.4% and bitterness 1.7% (Kirimura et al., 1969).

In Oriental countries, some natural seasoners such as dried bonito and certain species of mushroom such as Shiitake (Lentinus edodes) have been known to contain substances imparting flavor to food. 5'-Nucleotides such as disodium 5'-inosinate and disodium 5'-guanylate have been found to be the main components of the taste of these natural seasoners. Kuninaka et al, found that a small amount of nucleotide can synergistically increase the strength of the taste of MSG. The taste intensification resulting from the addition of MSG to foods is a consequence of the flavor contributory effect of MSG by itself and also of the synergism between 5'-nucleotides in foods and added MSG (Kuninaka et al., 1964). Several investigations were undertaken to obtain evidence of the validity of a "sentilization" hypothesis that MSG does not impart a flavor of its own but serves only to enhance the natural flavors of foods by increasing the sensitivity of the taste receptors (Cairncross et al., 1948; Mosel et al., 1952; Pilgrim et al., 1955).

A number of factors had some influence on effectiveness of the addition of glutamate, and the inherent level of free glutamate in the product had a major influence on the effectiveness of added glutamate. Glutamate is normally used as a supplement to foods containing salt. A consumers' preference test showed that the extent of preference for the glutamate was apparently influenced both by the product and salt contained in it (Hanson et al., 1960a).

Effects of MSG and its uses in foods and food products have been reported as follows: Flavor was improved in such foods as meats, seafoods, stews, soups, chowders and cooked vegetables (Cairncross et al., 1948); raw vegetables, cooked fresh vegetables, canned vegetables and cooked frozen vegetables (Sjostrom et al., 1948); clam and fish chowder, fish cakes, soups and purees (Fellers, 1948); canned vegetables, frozen and dehydrated vegetables, meat, poultry and fish recipes (Girardot et al., 1954), fried chicken, chicken patties and canned chicken (Rogers et al., 1956), and seasoned sausage after storage (Kemp, 1955). A flavor stabilizing effect of MSG in frozen foods has been studied and discussed (Norton et al., 1952 ; Hanson et al., 1960b).

Although glutamate has been studied in a wide variety of foods in which its beneficial effects have been demonstrated, glutamate appears to be ineffective in others. Fruits and fruit juices contain measurable quantities of free glutamate (Fernandez-Florez 1970), however, glutamate appears to be not effective in enhancing the flavor of many fruit based products. Similarly, sweet baked goods, dairy

products and cooked cereals are not considered to be benefited by use of MSG. (Cairncross et al., 1948).

Biochemical Data

BIOCHEMICAL DATA

1. Occurrence

L-Glutamic acid is a common component of animal and plant proteins and is also widely distributed in the living organism as free glutamate. A number of studies have been made on the distribution of glutamate in many kinds of foods, as shown in Table 1.

Human milk contains 11.5-30.6 mg/100ml of free glutamate (Stegink et al., 1972), while cow's milk contains 4 mg/100 gm (Mueller, 1970). Human plasma contains 0.44-0.48 mg/100ml of free glutamate and 10.8 mg/100ml of bound glutamate (Peters et al., 1969). Plasma of monkey, rat , mouse, cat and pig contains 0.88-2.21 mg/100ml (Boaz et al., 1974), 1.54 mg/100ml (McLaughlan et al., 1970), 1.15 mg/100ml (Filer et al., 1973), 1.7-2.8 mg/100ml (Waelsch, 1949) and 1.38 mg/100ml (Stegink et al., 1973b) of free glutamate respectively. Human spinal fluid contains 0.034-0.246 mg/100ml (mean 0.103) of free glutamate (Dickinson et al., 1966). Human urine contains 2.1-3.9 μg of free glutamate /mg of creatinin and 200 μg of bound glutamate /mg of creatinin (Peters et al., 1969).

2. Chemical and physical properties

Definition of MSG

Chemical names: Monosodium L-glutamate monohydrate, 2-Aminopentanedioic acid monosodium salt monohydrate.

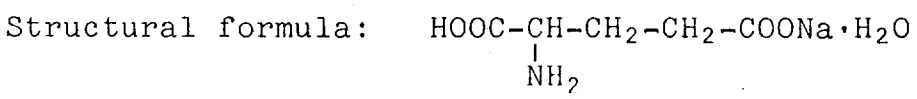
Table 1. The glutamate content in foods

Product	Total Glutamate* (g/100g)	Free Glutamate (mg/100g)	Reference
Milk			
Fresh milk	0.819	4	Müller, 1970
Condensed milk	–	14	Müller, 1970
Milk Products			
Casein	23.052	–	
Buttermilk	0.620	–	
Cheese			
Cammembert	4.787	–	
Parmesan	9.847	1303-2170	Müller, 1970
Poultry Products			
Eggs	1.583	23	Müller, 1970
Chicken	3.309	44	Maeda et al., 1958
Duck	3.636	69	Madea et al., 1961
Meet			
Beef	2.846	33	Maeda et al., 1958
Lamb	2.730	20	Hac et al., 1949
Pork	2.325	23	Maeda et al., 1958
Fish			
Cod	2.101	9	Maeda et al 1961
Mackerel	2.382	36	Maeda et al., 1958
Salmon	2.216	20	Hac et al., 1949
Vegetables			
Peas	5.583	190	Hac et al., 1949
Beets	0.256	30	Hac et al., 1949
Carrots	0.218	33	Maeda et al., 1958
Onions	0.208	21	Maeda et al., 1958
Spinach	0.289	39	Maeda et al., 1958
Tomatoes	0.238	140	Maeda et al., 1958
Tomato juice	–	270	Müller, 1970
Tomato juice, concentrated	–	370	Müller, 1970
Fruits			
Apple	–	0.9	Fernandez-Flores et al., 1970
Banana	–	36.7	Fernandez-Flores et al., 1970
Cantaloup	–	54.3	Fernandez-Flores et al., 1970
Grapefruits	–	36	Müller, 1970
Orange	–	13.1	Fernandez-Flores et al., 1970
Watermelon	–	29.1	Fernandez-Flores et al., 1970
Grains			
Bread, white	2.952	–	
Corn	1.765	120	Hac et al., 1949
Rice	1.041	–	

* Reference (Orr et al., 1957)

Chemical formula: $C_5H_8NNaO_4 \cdot H_2O$

Structural formula:



Molecular weight: 187.13

MSG occurs as practically odorless, colorless to white, prismatic crystals, or a white crystalline powder, having a characteristic taste. It is freely soluble in water, sparingly soluble in alcohol and practically insoluble in ether (US Committee on Specifications, 1972, 1974; Japanese Union of Food Additives Associations, 1974; FAO/WHO, 1971b). Its melting point is 195°C and specific gravity is 1.635 (Chem. Soc. Japan, 1966).

MSG is scarcely hygroscopic, so it will not change both in appearance and quality during storage. The characteristic taste of MSG is stereochemically assumed to be attributed to the molecular structure itself. The D-Isomer of monosodium glutamate does not possess a characteristic taste or enhance flavors. (Kaneko, 1939).

Heating under food processing or cooking scarcely causes any decomposition of MSG. For example, when 0.2% aqueous solution of MSG, containing 2% of sodium chloride at pH 5.6, is heated at 100°C for one hour, the amount decomposed is only 0.6% (Motozaki, 1969a). Under strongly acid conditions at high temperature, a small portion of MSG is dehydrated and converted into pyrroglutamate (5-pyrrolidone-2-carboxylate) (Motozaki, 1969b).

The racemization of MSG to monosodium DL-glutamate tends to occur in strongly acid (below pH 3) or alkaline (above pH 8.5) conditions, especially in the latter. The racemization is promoted by heating under the above conditions and results in a decrease of the characteristic taste of MSG. Maillard (or browning) reaction tends to occur when MSG is treated at high temperature with a large amount of reducing sugars (Motozaki, 1969a).

3. Specifications

Analytical data for the three sets of international specifications for MSG are shown in Table 2.

There are no numerical limitations on impurities other than described in the specifications. However, special analyses for MSG do not indicate the presence of the chemical carcinogens; polycyclic aromatic hydrocarbons, phenolics and nitrosamines in MSG. In these analyses detection limits were 1 ppb, 0.5 ppb and 5 ppb respectively (Burrows et al., 1970). The specifications for glutamic acid, glutamic acid hydrochloride, monoammonium glutamate and monopotassium glutamate are described in Food Chemicals Codex (US Committee on Specifications, 1972).

Table 2. Specifications for MSG

	FCC[1]	JSFA[2]	FAO/WHO[3]
Content of $C_5H_8NNaO_4 \cdot H_2O$[4]	>99.0%[5] (after drying at 100°C for 5 hours)	>99% (after drying at 100°C for 5 hours)	>99% (after drying at 97-99°C for 5 hours)
Clarity and color of solution	passes test[6]	passes test[7]	———
Specific rotation	$[\alpha]_D^{20}$ +24.8 ~ +25.3°[8]) $[\alpha]_{546.1m\mu}^{25}$ +29.7 ~ +30.2°[9])	$[\alpha]_D^{20}$ +24.8 ~ +25.3°[8])	$[\alpha]_D^{20}$ +24.8 ~ +25.3°[8])
pH of aqueous solution	6.7 - 7.2 (5% solution)	6.7 - 7.2 (10% solution)	6.7 - 7.2 (5% solution)
Limit of impurities	———		———
Ammonium		< 0.02% (as NH_4)	
Arsenic	< 3ppm (as As)	< 2ppm (as As_2O_3)	< 2ppm (as As)
Chloride (as Cl)	< 0.2%	< 0.07%	< 0.2%
Heavy metals (as Pb)	< 20ppm	< 10ppm	< 10ppm
Lead (as Pb)	< 10ppm	———	< 5ppm
Loss on drying	< 0.5% (when dried at 100°C for 5 hours)	< 0.5% (When dried at 100°C for 5 hours)	< 0.5% (when dried at 97-99°C for 5 hours)
Other amino acids	———	chromatographycally not detectable[10])	———

1) Food Chemical Codex, 2nd ed. (US Committee on Specifications, 1972, 1974)

2) The Japanese Standards of Food Additives, 3rd ed. (Japanese Union of Food Additives Associations, 1974)

3) FAO/WHO Food Additive Specifications (FAO/WHO, 1971b)

4) Analytical method: non-aqueous titration

5) > A means not less than A; < B means not more than B

6) A 1 in 10 solution of the sample in water is colorless and has no more turbidity than the control

7) A 1 in 10 solution of the sample in water is colorless and clear

8) Determine in a solution containing 10g of the sample in 100 ml of 2*N* HCl (FCC); 2.5*N* HCl (JSFA); 7% HCl (FAO)

9) Determine in a solution containing 15g of the sample in 100 ml of 2.3*N* HCl

10) Paper chromatography, 10µg of the sample, coloration by ninhydrin spray, only one spot is visible

BIOLOGICAL DATA

1. Biochemistry

1-1. Metabolism

Glutamic acid is not classified as an essential amino acid, but it plays a role as a necessary amino donor for the synthesis of other amino acids. The normal metabolic steps of glutamate including oxidative deamination, transamination, decarboxylation and amidation (Kergl et al., 1954 ; Von Euler et al., 1938) are well established in mammals.

Oxidative deamination of glutamate to α-ketoglutarate is catalysed by glutamate dehydrogenase [E.C. 1.4.1.3] (Von Euler et al., 1938 ; Cohen, 1949). Glutamate is also converted into α-ketoglutarate by transaminase (GOT)[E.C. 2.6.1.1], GPT[E.C. 2.6.1.2]) which catalyses the reversible transfer of amino substituents of glutamic acid to α-keto acids (Cohen, 1949).

The main catabolic steps of glutamate in mammals involves deamination or transamination followed by oxidation of the resulting α-ketoglutarate in the TCA cycle (Meister, 1965).

Decarboxylation of glutamate to γ-aminobutyrate by glutamate decarboxylase [E.C. 4.1.1.15] is a significant pathway of glutamate metabolism in mammalian brain (Roberts et al., 1950 ; Roberts et al., 1951) and recently, the reaction has been shown to occur in several other non-neuronal mamalian tissues (Whelan et al., 1969 ; Scriver et al., 1969).

Glutamine formation from glutamate and free NH_3 is known to be catalysed by glutaminase [E.C. 3.5.12] (Krebs, 1935 ; Speck, 1949), which is designated as the brain type one (glutaminase I) to distinguish the enzyme from the liver type (glutaminase II system) which involves glutamine α-ketoacid transaminase in its system (Greenstein, 1947). Recently, glutaminase I has been shown to have characteristics of both allosteric and polymeric proteins (Svenneby et al., 1970).

An extenisve study on exogenous monosodium L-glutamate metabolism has been carried out on neonatal pigs using a labeled compound. When U-^{14}C-monsodium glutamate (1 g/kg body wt.) was intubated to 3-day-pigs, label was rapidly incorporated into plasma glutamate, glutamine, arginine, aspartate, alanine, ornithine, citrulline, urea, and also into plasma glucose and lactate, with 65-80% in glutamate, glucose, and lactate, of which glutamate was most rapidly removed from the plasma. ^{14}C was found in plasma pyruvate and α-ketoglutarate, but not in succinate, malate, oxaloacetate, or pyrrolidone carboxylate. Neither labeled glutamate nor aspartate entered the spinal fluid as such, although label was found in spinal glutamine, glucose, lactate, and urea. The available pathways of exogenous glutamate metabolism as shown in Fig. 1 was proposed (Stegink et al., 1973a).

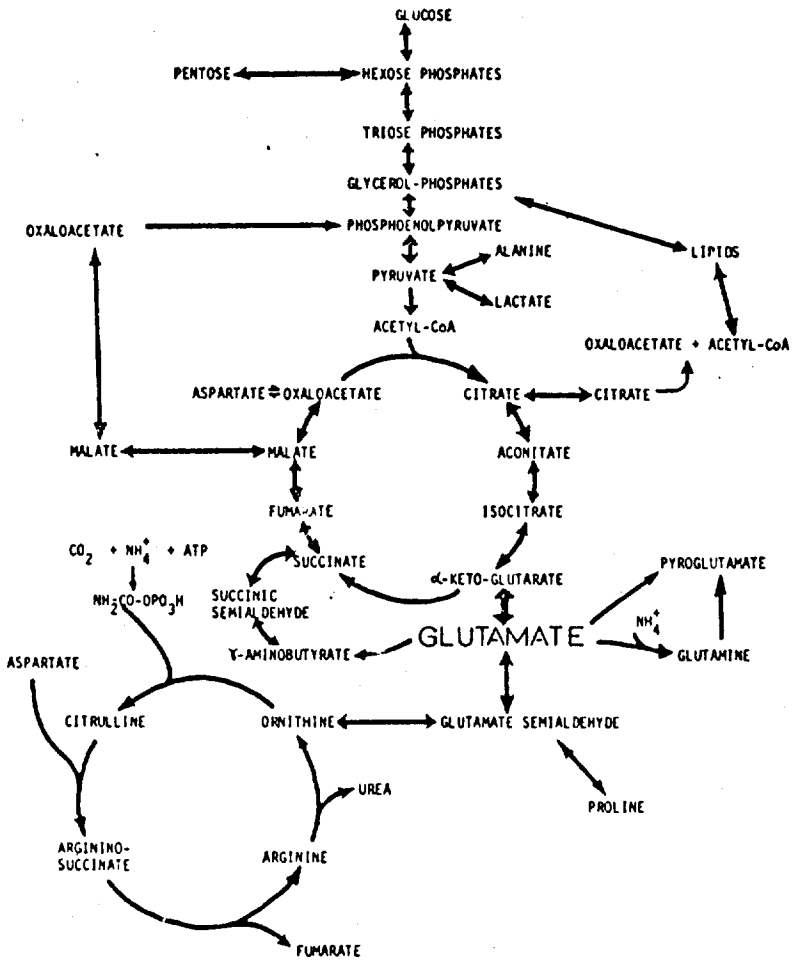


Fig. 1. Available pathways of exogenous glutamate (Stegink et al., 1973a)

Many other similar studies have been made by using different routes of administration, animal species, or organs. Main experiments are as follows: in rat brain and liver mitochondrial systems (studies *in vitro* by using [1-^{14}C] and [5-^{14}C]-glutamate) (Balázs, 1965), in the isolated rabbit heart (perfusion study *in situ* by using U-^{14}C-glutamate) (Doell et al., 1959), in cerebral cortex of male cats ranging in age from 1 week through adulthood

(study on compartmentation of glutamate metabolism *in vivo* by using ^{14}C-L-glutamate and ^{14}C-L-aspartate) (Berl, 1965), in swiss albino mice weighing 24-26 g (study on compartmentation of glutamate metabolism in brain *in vivo* by using [1-^{14}C]- and [2-^{14}C]-glucose, and [1-^{14}C]- and [2-^{14}C]-acetate) (Van Den Berg et al., 1969), in male albino rats (metabolism of 2-^{14}C-L- and 2-^{14}C-D-glutamate, 5-^{14}C-DL-glutamate and 5-^{14}C-D-glutamate given by p.o. administration or intubation into cecum) (Wilson et al., 1961), in rats and mice (study on i.v. injected U-^{14}C-glutamate uptake into organs and its distribution among metabolites) (Lajtha et al., 1959), in the lactating cow (studies on utilization of i.v. injected ^{14}C-glutamate) (Egan et al., 1968) and goat (Egan et al., 1970). These results are essentially consistent with those of Stegink mentioned above.

1-2. Absorption

Recently, absorption including glutamate and other metabolite levels of blood and by administration of relatively large amount of exogenous glutamate have been studied by many researchers.

1) Human

The oral administration of monosodium L-glutamate (1 g/10kg body wt.) to 4 human subjects led to an increase of the glutamate levels in the peripheral blood in various magnitudes from control levels of 0.6-1.2 mg/100ml to

maximal levels of 1.0-9.5 mg/100ml (Bessman et al., 1948). When glutamic acid compounds equivalent to 15 g of the glutamic acid were given with tomato juice or water to adult human subjects, little absorption occurred with the unneutralized glutamic acid and the hydrochloride, but the neutralized salts were well absorbed, the blood level rising in 1 hour from 2 mg/100ml to 35 mg/100ml (Himwich, 1954).

The oral administration of sodium glutamate (0.1 g/kg body wt.) to lactating women resulted in small increases in plasma glutamate from control levels of about 4 μmoles/100ml to maximum levels of about 13 μmoles/100ml with large variation among subjects. However, little change was noted in breast milk amino acid levels (Stegink et al., 1972).

2) Animals

Blood and brain glutamate levels in young rats given monosodium L-glutamate alone or with food were studied. Plasma glutamate rose rapidly to a peak (approx. 12 mg/100ml) from a normal level (approx. 3.3 mg/100ml) in 20 minutes when monosodium L-glutamate (0.2 g/kg) was given alone and more slowly to a peak in 30 minutes when given with veal (2 g/kg). A linear relationship was noted between the plasma level of glutamate and doses above 0.2 g/kg body weight of monosodium L-glutamate. After moderate oral doses (below 0.2 g/kg), comparable to human intakes, blood levels remained normal (approx. 10 mg/100ml). Subcutaneous injection of monosodium L-glutamate (0.5 g/kg body wt.) produced the same plasma

levels (approx. 15 mg/dl) as that produced by oral feeding. Brain levels of glutamate (10-15 µmoles/g tissue) were not affected with administration of monosodium L-glutamate (0.5 mg/g body wt., s.c. or 0.1-2.5 mg/g body wt., p.o.) (McLaughlan et al., 1970).

Many other similar observations have been conducted by using a variety of doses, different routes of administration, and species as follows: in male rats and mice (MSG 0.5-8 g/kg body wt. p.o. for adults, and 0.5-4 g/kg body wt. p.o. for neonates) (Ichimura, 1970c), in young mice (50 µl/g body wt. with a 5% casein hydrolysate-5% dextrose solution = 0.2 mg of MSG + Aspartate/g body wt. s.c.) (Filer et al., 1973), in pregnant rats on day 19 of gestation (MSG 8 g/kg body wt. p.o.) (O'hara, 1970), in guinea pigs of both sexes weighing 300-550 g (20 mmoles/kg body wt., with diet) (Christensen et al., 1948), in dogs weighing 12 and 20 kg (introduction of 0.15-10% monosodium L-glutamate solution into the small intestine) (Neame, 1957), and in neonatal pigs 3 days of age (MSG 0.01-1 g/kg body wt. with or without food, p.o.) (Stegink et al., 1973b).

Effects of dietary glutamate on some brain and liver metabolites in rats were studied. Feeding of monosodium L-glutamate (1, 5, 10 and 20% in diet, *ad lib*) for 16 weeks resulted in no changes in levels of glutamate (9.2 µmoles/g), aspartate (2.43 µmoles/g), DNA (1.18 mg/g) and protein (114 mg/g), and glutamate decarboxylase in brain. But GABA

levels in brain decreased significantly from 2.59 μmoles/g tissue with a significant increase in succinate level (0.401 to 0.508 μmoles/g tissue) after feeding of 20% monosodium L-glutamate. In liver, dietary monosodium L-glutamate had no effect on the contents of protein (202 mg/g), RNA-p(0.699 mg/g), DNA-p(0.106 mg/g), glutamate (1.58 μmoles/g), lactate (11.25 μmoles/g), malate (0.53 μmoles/g), or α-GPO_4 (0.94 μmoles/g) but aspartate levels were significantly increased from 0.44 μmoles/g to 0.58 μmoles/g by feeding of 20% MSG (Prosky et al., 1971). Similar results were noted in second generation neonatal rats born to parents fed a diet supplemented with 10% monosodium L-glutamate (Prosky et al., 1972).

Monosodium L-glutamate (2 mg/g body wt.) was subcutaneously injected into 4-day-old mice. Blood glutamate concentrations peaked rapidly, reaching a maximum of 40 mM within 15 minutes but returned precipitously to near-baseline values (below 1 mM) in 1-3 hours after injection. Glutamate levels in the arcuate nucleus steadily increased to reach a peak value of 110.9 mmoles/kg dry wt. at 3 hours following the injection, whereas the highest levels reached in the ventromedial hypothalamus or lateral thalamus were about 41.7 mmoles/kg dry wt. Return to control values of about 25 mmoles/kg dry wt. occurred gradually over a period of 12-15 hours in all three brain regions. Possible mechanisms were discussed to account for the relation between the

transient marked accumulation of glutamate in arcuate nucleus and the selective destruction of arcuate neurons (Perez et al., 1972). Monosodium L-glutamate load (1-4 g/kg body wt.) to neonatal monkeys resulted in a rapid and extreme elevation of plasma levels, with peak elevations (200-450 μmoles/dl, from normal levels of 6-15 μmoles/100ml) roughly proportional to the dose administered. Plasma aspartate levels were also elevated, but all the other amino acids were unchanged. No animals studied showed signs of massive hypothalamic lesion (Boaz et al., 1974).

1-3. Effect on enzyme activity and fat and sugar metabolism

Recently, studies of information about the effects of exogenous glutamate on some glutamate metabolizing enzymes and other related enzymes have been accumulated.

An even (3-4 times) greater increase in the specific activity of this enzyme was noted in fetal liver slices of rat and human *in vitro* when these slices were incubated with 200 mg/ml of potassium glutamate (Francesconi et al., 1968). Hepatic GOT and GPT activities in rats and mice have been shown to increase characteristically with the development of animals (1-100 days of age). It was also shown that there was a marked correlation between hepatic GOT activities and plasma glutamate levels of rats and mice dosed orally with 1 g/kg body wt. monosodium L-glutamate (Hashimoto, 1970). Specific activities of hepatic carbamyl phosphate synthetase have been shown to increase markedly during

3 consecutive weeks of p.o. administration of monosodium L-glutamate (4 g/kg body wt./day) and to return toward control values by prolonged (more than 6 weeks) administration of glutamate, indicating adaptation of this enzyme to the administered glutamate (Hutchinson et al., 1965).

Effects of large doses of monosodium L-glutamate on glutamate metabolizing enzymes in rat retina, brain and liver were studied in connection with the retinal lesion by the s.c. administration of glutamate. Approximately a 40-50% decrease in retinal glutaminase I activity and 30% increase in retinal GOT activity by s.c. injection of glutamate (2.2-5.5 g/kg) were noted with glutaminase II. Glutamosynthetase and glutamotransferase activities in retina were not changed by treatment. It was suggested that the retinal lesion in rats might be associated with a reduction in the level of retinal glutaminase I due to a product (glutamate) repression. There was no evidence that they were causally related to the injury (Freedman et al., 1962 ; Freedman et al., 1963).

Subcutaneous injection of monosodium glutamate (1 g/kg body wt.) or monosodium aspartate (0.92 g/kg body wt.) to new born mice (1 day of age) resulted in 2-3 fold increases in activities of GDH, GOT and GPT in the brains and livers of new born mice. However, the injection resulted in no significant effect on these enzymes in the brain and liver of weanling mice (Arthur, 1973).

Intravenous injection of neutralized glutamic acid hydrochloride (20 g) into hypoglycemic human subjects resulted in a rise in blood glucose (approx. 22 mg/100ml) compared with the levels (approx. 13 mg/dl) of subjects treated with NaCl. Glutamate i.v. injection to normal subjects failed to cause any significant effect on blood glucose (Mayer-Gross et al., 1949).

When the dose of i.p. injected glutamate to rats weighing 200-300 g was raised from 0.01 to 0.20 mmoles/100g body weight, plasma glucose concentration increased from 88 to 173 mg/100ml. And the increase in plasma glucose level could not be explained by stoichiometric conversion of exogenous glutamate to glucose (Marcus et al., 1967).

When the 8 essential amino acids plus glutamate (137 g/day) as a source of nonessential nitrogen (total N = 16 g) were fed to 8 healthy male human subjects (19-55 years of age) for 3-4 weeks, however, serum cholesterol markedly fell by 37 mg/dl, phospholipid 19 mg/dl, and β-lipoproteins 73 mg/dl. Triglycerides actually increased by 49 mg/dl (Olson et al., 1964). The hypolipidemic effects of glutamate and saturated fatty acids in the diet in human were not additive, suggesting that these two dissimilar agents affect a final common pathway in the metabolism or distribution of serum lipids (Olson et al., 1970). These hypocholesteremic effects of glutamate as observed in humans occurred in Mongolian gerbils and chicks but not on rabbits or rats (Bazzano et al., 1969 ; Bazzano et al., 1970).

2. Pharmacology

There are few reports on pharmacological studies of exogenously administered monosodium L-glutamate (MSG) to humans except recent reports on the effects of the so-called "Chinese Restaurant Syndrome". There are references to the use of glutamate in treatment of mental retardation in humans.

Several reports indicated that L-glutamate is a good transmitter candidate in the central nervous system (Krnjevic, 1970 ; Johnson, 1972).

L-Glutamate applied iontophoretically to the external surface membrane of neurones within the spinal cord of anesthetized cats excited interneurones, Renshaw cells and motoneurones by producing a membrane depolarization. This action was considered non-specific and unrelated to that of an excitatory synaptic transmitter (Curtis et al., 1960). L-Glutamate and several related amino acids were applied to single units in the cerebral and cerebellar cortex of cats, rabbits and monkeys under various conditions of anesthesia. These substances excited all neurones quickly and powerfully (Krnjevic et al., 1963). L-Glutamic acid and related acidic amino acids were administered electrophoretically into the extraneuronal environment of single neurones in the pericruciate cortex of anesthetized cats. These amino acids excited cortical neurones (Crawford et al., 1964).

In cats given intravenously 50-100 mg/kg of MSG, electroencephalograms revealed a functional acceleration tendency. In rats injected intracranially 50 or 100 mg/kg of MSG, the EEGs showed the same tendency as in cats. The tendency of functional acceleration was due to the influences on a reticular formation of the brain stem (Hara et al., 1962). However, high doses (0.69, 1.38 g/kg) of L-glutamic acid by the intravenous administration did not cause any demonstratable change in the EEG of dogs (Herbst et al., 1966).

Intraventricular injection of L-glutamate caused excitation at a dose of 150 µg but no effect at 50-100 µg in 4-week-old mice (Crawford, 1963). L-Glutamic acid injected intracisternally in Wistar rats weighing 130 to 200 g caused various seizures according to the dose. Low doses caused running seizures and automatism (CD_{50} : 3.6 mg/kg). Medium doses caused clonic jerks of the forelegs (CD_{50} : 4.9 mg/kg). High doses caused generalized tonic-clonic seizures (CD_{50}: 7.0 mg/kg) (Hennecke et al., 1970).

MSG (0.5 M solution, 50-250 µl) stereotactically injected into different cerebral regions of cats caused generalized electroencephalographic seizure. The hippocampus was shown to have the lowest seizure threshold of all regions investigated. Other cerebral regions and the ventricle did not react at all or reacted only to higher concentration of MSG (Knaape et al., 1970).

One hundred and ninteen Sprague-Dawley male rats (9-12 weeks old) were given intraperitoneally 20 mmoles (about 3.4 g)/kg of MSG. Marked somnolence was observed in 99% of the animals within 5-10 min. About half of the animals salivated copiously and 31% displayed spastic tremors varying in intensity from mild to severe myoclonic jerking sometimes followed by vigorous running around the cage and stereotyped biting. Seizures occurred in 17%, and 29 of total 119 rats died during 2 hours after injection of MSG (Bhagavan et al., 1971). An electroencephalographic study on the above mentioned rats was also reported (Stewart et al., 1972).

MSG (1-4 g/kg) or several amino acids were given intraperitoneally or orally to infant Holtzman rats. Convulsions observed after these doses were not unique to MSG: monosodium L-aspartate gave similar symptoms. Equimolar doses of glycine did not cause convulsions. MSG caused an increase in brain glutamine but not in brain glutamate. Monosodium L-aspartate or glycine also increased brain glutamine. The authors suggested that MSG induced convulsions were not due to ammonia but to the amino acid anions (Mushahwar et al., 1971). Infant rats (10-day-old, weighing 16-23 g) and adult rats (13-week-old, 180-200 g) were given intravenously MSG and related excitant amino acids (0.5-20 mmoles/kg). MSG produced no apparent effects up to 3 hours after injection at doses of 10 mmoles/kg but produced convulsions about 30 min. after at 20 mmoles/kg in infants.

None of the excitant amino acids produced convulsions in adults (Johnston, 1973).

Some behavioral studies on MSG treated animals have been reported.

Infant litter mates of Swiss-Webster mice were divided into two groups. The experimental group received single daily subcutaneous injection of MSG for 10 consecutive days. The control received the same volume (0.02 ml) of 0.9% saline. The first MSG injection, started 24 hours after birth, contained 2 g/kg of MSG. Subsequent doses were in ascending daily increments of 0.25, up to 4.25 g/kg/day, through ten days. A battery of behavorial and pharmacological tests were applied, when these mice attained 20-28 g body weight. There were no significant or observable differences in their responses to behavioral tests such as spontaneous motor activity, test of neurological defect, grip-strength endurance test and swimming endurance test. Similarly, no differences were noted in the responses of these two groups to drugs such as amphetamine, pentylenetetrazol and hexobarbital (Prabhu et al., 1971).

Male weanling rats received diets containing MSG and monopotassium L-glutamate (MKG), each 10% or NaCl and KCl, each equivalent to the cation in the glutamate salt, respectively for approximately 34 weeks. Pharmacologic measurement were made after 24 weeks on these diets.

Motor activity was unchanged in all substances, Amphetamine-induced stimulation of motor activity was normal in MSG or NaCl treated rats but was reduced in MKG or KCl treated rats. Tests of learning deficits in an avoidance-escape situation indicated that animals receiving MSG failed to develop avoidance lever-pressing behavior. This effect was also seen in some NaCl treated rats. In contrast, animals treated with MKG and especially KCl showed a high rate of avoidance responding (Weiss et al., 1971).

Five groups of 10 male Holtzman albino weanling rats were given 0, 1, 5, 10 and 20% MSG in the diet for 16 weeks. Hyperirritability, an excessive response to handling, was observed in all groups of rats consuming diets supplemented with MSG (Prosky et al., 1971).

Eight litter mates from each of ten mother Holtzman rats were divided into 4 groups consisting of 2 litter mates from each mother. These groups were orally given water and 1.25, 2.5 or 5 g/kg of MSG, respectivity, all in volumes of 10 ml/kg, daily from 5 to 10 days of age. Rats were placed in separate cages and were subjected to 3 different behavioral tests at 3 months of age. Spontaneous motor activity and deficiency in discrimination learning as in a maze experiment were normal in both groups treated with 1.25 and 2.5 g/kg MSG but less activity was seen in the 5 g/kg treated group. Learning of a fixed-ratio food reinforcement schedule was, however, not affected (Pradhan et al., 1972).

The effects of MSG (5 and 10 g/kg, orally) on avoidance behavior were studied in Wistar rats weighing 200-250 g. Only a higher dose of MSG was effective in depressing avoidance acquisition and performance (Pinto-Scognamiglio et al., 1972).

In rabbits anesthetized with urethane, intravenous injection of MSG (up to 1 mg/kg) had no influence on respiration and blood pressure. High doses of MSG (100-800 mg/kg) produced transient respiratory stimulation and hypertension. MSG had little or no effect upon isolated rabbit intestine (Hara et al., 1962).

3. Toxicology

3-1. Acute toxicity

LD_{50} values of monosodium L-glutamate (MSG) and related compounds in mice, rats, chicks and rabbits are summarized in Table 3.

3-2. Subacute and chronic toxicity

There are a number of subacute and chronic toxicity studies in various animal species such as mice, rats, rabbits, beagle dogs and monkeys. The results of the studies are summerized in the following.

1) Male C-57/Black strain mice 6 weeks of age were used in this study. A control group of 200 animals and 6 treatment groups of 100 animals received 0%, 1% or 4% in the diet of either monosodium L-glutamate, monosodium DL-glutamate, or L-glutamic acid for 2 years. The average daily intakes of the test substances in 1% and 4% treatment groups were 1.65 g/kg body weight and 6.60 g/kg body weight, respectively.
No adverse effects were noted on general physical condition, hematology, macroscopic examination or histopathology.
No malignant tumors appeared after 2 years that could be related to the administration of the test substances.
(Little, A.D. 1953b)

Table 3. Acute toxicity of monosodium L-glutamate and related compounds

Substance	Animal (Strain)	Sex	No. of animal in each dose	Route of Administration	LD_{50} mg/kg body weight (95% confidence limit)	References
Monosodium L-glutamate	Mouse (dd-strain)	Male	10	Oral	16200 (14200 - 18400)	Ichimura, et al. (1968)
	Mouse	—	—	Oral	19200 (22840 - 16130)	Pinto-Scognamiglio (1972)
	Mouse (dd-strain)	—	—	i.p.	6900	Yanagisawa, et al. (1961)
	Rat	—	—	Oral	16600 (18900-14500)	Pinto-Scognamiglio (1972)
	Rat	—	—	Oral	19900	Little, A.D. (1953a)
	Rat	—	—	i.p.	3600	Klingmüller, et al. (1955)
	Chick (Broiler)	—	16	s.c.	3000 - 4000	Carew, et al. (1971)
L-Glutamic acid	Rat		36	Oral	> 30000	Little, A.D. (1953a)
	Rabbit		13	Oral	> 23000	Little, A.D. (1953a)
Monosodium DL-glutamate	Rat		57	Oral	10300	Little, A.D. (1953a)
Monoammonium L-glutamate	Rat			i.p.	1000	Klingmüller, et al. (1955)

2) Sprague-Dawley rats of 3 months of age were administered monosodium L-glutamate (L-MSG), monosodium DL-glutamate (DL-MSG) or L-glutamic acid (L-GA) for 2 years. The levels of substances in the diet and number of animals are indicated below:

Supplement	Number of Animals Males	Females	Total
0.1% L-MSG	40	35	75
0.4% L-MSG	35	40	75
0.1% DL-MSG	40	35	75
0.4% DL-MSG	35	40	75
0.1% L-GA	40	35	75
0.4% L-GA	35	40	75
Control Diet	61	89	150

No adverse effects were noted on body weight change, food consumption, general physical condition, behavior, survival rate, fertility, hematology, gross examination and histopathology, or tumor incidence in any treatment groups (Little, A.D. 1953a).

3) One control group and 9 treatment groups of 5 male rats of Wistar strain were given by gastric tube natural monosodium L-glutamate, synthetic monosodium L-glutamate or synthetic monosodium D-glutamate in amounts of 20, 200 and 2000 mg/kg body-weight once a day for 90 days respectively. No adverse effects were noted on physical appearance, food and water

consumption, body weight change, volume and weight of the cerebrum, cerebellum, heart, lungs, stomach, liver, spleen, and kidneys. No histological changes in internal organs were found by macroscopic and microscopic examination (Hara et al., 1962).

4) Diets containing monosodium glutamate (MSG) and monopotassium glutamate, each 10%, or sodium chloride and potassium chloride, each equivalent to the cation in the glutamate salt, were given to groups of male weanling rats for approximately 34 weeks. After 12 and 24 weeks, animals receiving MSG showed reductions in weight gain as a group significantly different from controls, while groups receiving the other substances had smaller changes in this respect. Food consumption was similar in all groups (Weiss et al., 1971).

5) Six groups of 6 weanling male Charles River CD rats were fed 100 g basal diet plus either 20 g glucose, 20 g monosodium L-glutamate (MSG), 40 g MSG, 17.4 g L-glutamic acid, or sodium salts plus nonessential amino acids (the same quantity of sodium and nonessential amino acid nitrogen found in 20 g MSG) for 5 weeks. No mortality occurred. The growth depression observed in rats fed MSG diets appeared to reflect sodium intake more than glutamic acid consumption. Dietary sodium content was also a factor in kidney size, water consumption and urinary pH. No conclusive endocrine effect was found (Wen et al., 1973).

6) Groups of 18 male rats of Sprague-Dawley strain, weighing about 55 g, received for 3 weeks the diets in which amino acids such as L-glutamic acid, L-tryptophan, L-tyrosine, L-lysine, glycine, L-arginine, L-leucine and L-aspartic acid were added to the ground laboratory chow 7% or 10% by weight. Body weight gain, food intake, and food efficiency of the rats fed the diets added L-glutamic acid were essentially comparable to those of the rats fed the basal diet only (Daniel et al., 1968).

7) Three groups of rats were given 1, 2, or 4 g/kg of MSG as a single daily oral dose and one group received an iso-osmotic amount of NaCl. This experiment was carried out for 90 days. Body weight gain, hematologic values, blood chemical determinations, and urinalysis did not differ significantly between groups. A histologic appraisal of tissues taken at sacrifice showed no significant pathologic changes (Rosenblum et al., 1970).

8) Four groups of 16 male and 24 female rabbits of New Zealand White strain received ground Purina Rabbit Chow containing 0%, 0.1%, 0.825% or 8.25% monosodium L-glutamate for 6 to 7 weeks. No adverse effects which could be attributed to compound were noted on body weight gains, food consumption, appearance, behavior or gross pathology (Hazleton Laboratories, 1966).

9) Details of feeding studies in rats and mice and teratology studies in rabbits have been presented (Ebert 1970). The results indicated no toxicity due to chronic administration of MSG, however a high percentage of both treated and control groups showed swellings or nodules - all of which, for purposes of the experiment, were assumed to be tumors. Such an incidence of neoplasias was not surprising for Sprague Dawley rats but pointed to the need to carry out chronic feeding studies in an experimental species resistant to the formation of spontaneous neoplasias. Thus C57/Black mice were studied at ten times the levels studied in the rat namely 1% & 4% of the diet. No tumors were observed in MSG treated or control mice.

In teratologic evaluations the levels studied, up to 8.25% in the diet, were the equivalent of 30 mg/kg to 2.5 g/kg/day. No differences between treated and control groups were observed with respect to reproductive performance of teratologic manifestations. Similarly, there was no evidence of any effect on the hypothalamic arcuate nucleus of glutamate treated rabbits.

10) Four groups of 2 or 3 squirrel monkeys of 11 days of age were fed either a 0%, 4.8%, 9.1% or 16.7% monosodium L-glutamate (MSG) formula diet for 9 weeks. Three of the ten monkeys receiving MSG died, although two deaths appeared unrelated to treatment. The body weight gains of monkeys fed 0%, 4.8%, or 9.1% MSG were within the normal limits. However, the food intake and body weight of monkeys given 16.7% MSG were significantly depressed. Fasting plasma amino acids of all 4 groups showed no remarkable differences.

No abnormalities were found on blood pressure, electroencephalogram, electroretinograms or histopathology in all 4 groups (Wen et al., 1973).

11) One control group and one treatment group of one cynomolgus monkey and one bushbaby one month of age were fed a 9.1% monosodium L-glutamate (MSG) formula diet or a liquid diet containing no MSG for a period of 11 months. No adverse effects were noted for the animals of the treatment group. The body weight gains, EEG, ERG and plasma amino acid patterns were similar to controls (Wen et al., 1973).

In conclusion, dietary levels of approximately 10% or more of monosodium L-glutamate (MSG) or L-glutamic acid for an extended period of time may induce some effects such as reductions in body weight or organ weight changes in some animal species.

3-3 Reproduction and teratology

Reproduction and/or teratological studies of monosodium L-glutamate or L-glutamic acid were extensively carried out using mice, rats and rabbits. The following are summerized results of the studies.

1) Mice of IVCS and Swiss albino strains were used. Groups of 3 males and 3 females were maintained on diets containing

0%, 2% (=4g/kg body weight/day) or 4% (=8g/kg/day) monosodium L-glutamate. Mice were mated after 2 to 4 weeks on the test diet. Offspring (F_1) were weaned at 25 days of age, and fed the same diet as parents. At age 90 days, one litter selected from each group were allowed to produce offspring (F_2) of the second generation. Parent mice were maintained on test diets, for 100 days after delivery and F_1 mice for 130 days of age. F_2 mice were reared until 20 days of age. No effects were observed on growth, food intake, estrous cycle, date of sexual maturation (F_1 generation), organ weight, litter size, and body weight of offspring, and histopathology of major organs of parent and F_1 generation. Day of eye opening, general appearance and roentgenographic skeletal examination of F_2 generation showed no abnormalities (Yonetani et al., 1970).

2) Groups of 6 female Sprague-Dawley rats were fed diets containing 0.5%, 1% or 2% vitamin level. At each vitamin level diets contained monosodium L-glutamate (MSG) at 0%, 1% or 2%. Reproductive performance of the maternal rats and the offspring (1st generation), the size and growth of the litters and the survival rate of the offsping were studied. The addition of MSG to the diet resulted in an increased fertility rate, as well as increased survival rate at weaning of the offspring of the first generation, particularly at a 2% vitamin added level. Addition of MSG to the diet had no effect on growth rate in the neonatal period

and no significant differences were found in the individual average body weight at weaning. No females fed a higher MSG level showed any sign of obesity when they reached sexual maturity (Semprini et al., 1971).

3) Holtzman weanling rats were fed Purina laboratory chow alone or supplemented with 10% monosodium L-glutamate (MSG) for 100 days. The rats were mated on a one-to-one basis for 7-day period and the resultant offspring (first generation) after weaning were continued on the same diet as their respective parents for 10 days. At this time the first generation rats were mated and offspring of the second generation were obtained. No significant differences were noted in conception rate, pups per litter or body, brain and liver weights between offspring of the second generation of control and MSG fed rats during the first 21 days of postnatal development (Prosky et al., 1972).

4) Monosodium glutamate was administered orally in doses of 0.07, 0.7, or 7.0 g/kg/day to pregnant rats on days 6-15 or days 15-17 of pregnancy. No adverse effects were observed on the dam or the fetus, or on the subsequent growth of the young up to the period of weaning. Further physical development to maturity was also normal except that the progeny obtained from pregnant rats treated on days 15-17 of gestation showed impaired ability to reproduce (Khera et al., 1970).

5) Two female rats of Wistar strain were given 1 g (equivilant to 4g/kg/day) of monosodium L-glutamate once a day by stomach tube commencing at day one of pregnancy. There was no effect on pregnancy or lactation. Pups were divided into 3 groups. Two groups of 4 male and 4 female pups were nursed by parents receiving monosodium L-glutamate and one group of 2 males and 6 females by untreated parent. At weaning, one group of pups that had been nursed by a parent receiving monosodium L-glutamate received approx. 5g/kg monosodium L-glutamate daily for 220 days. All pups developed normally, and no abnormalities were noted in growth rate, time of sexual maturity, estrous cycle and fertility. Parents received 1 g of monosodium L-glutamate daily for 336 days and no effects were observed on growth or estrous cycle (Suzuki et al., 1970).

6) Male and female rats of Charles River CD strain were fed the diet in which 2% L-glutamic acid was added to Purina Laboratory Chow for 3 days before mating and pregnant females were fed the diet for the subsequent gestation period. On the 21st day the animals were delivered by hysterotomy. No essential differences were noted on number of offspring, mortality at birth, mean litter size, mean fetus weight, resorption sites per litter, pregnancy rate or visceral and skeletal anomalies of the fetus between the treated group and control group (McColl et al., 1965).

7) Three groups of 5-6 male and 5-10 female rats of the Wistar strain received by oral intubation daily of 25 mg/kg or 125 mg/kg body weight of L-glutamic acid monohydrochloride, or 2 ml of water (control). Administration of the test compound was carried out once a day in two consecutive periods; the first was 15 consecutive days prior to mating and the second 18 consecutive days of various stages of gestation. About 30 days after the last treatment all parents were sacrificed. No adverse effects were noted on weight gains, food intake or sexual cycles of females. No organ weight changes were seen in females but males on the higher dose level had enlarged spleens. Offspring of the first generation and the second generation showed no gross internal and external abnormalities (Furuya, 1967).

8) Four groups of 24 female and 16 male New Zealand white rabbits were fed ground Purina Rabbit Chow containing 0%, 0.1%, 0.825% or 8.25% monosodium L-glutamate 2 to 3 weeks prior to mating and were mated to animals within the same group. Females received the same diets until the 29th or 30th day of gestation. At this time all females were sacrificed and the fetus immediately delivered by Caesarean section. No significant differences between the control and MSG-treated groups were noted on body weight gains, food consumption, general appearance, survival, breeding performance, and all litter data including total number, number of live young, number of dead young, number of resorptions, gross internal and external abnormalities, and skeletal staining were normal (Hazleton Laboratories, 1966).

9) L-Glutamic acid hydrochloride in a dose of 25 mg/kg was given orally to female rabbits once a day for a period of days 1-15 of gestation, monosodium L-glutamate in the same dose and for the same period of time to 9 pregnant rabbits and saline solution or nothing to 11 pregnant rabbits which served as a control group. No differences were noted between the treated groups and the controls as to rate of conception, mean litter size, and nursing rate. The average body weight of the young at 30 days of age in the treated groups was slightly lower as compared with the control group, but the weights of testes, ovaries and adrenal glands in the young and ovaries, adrenal glands, liver, kidneys and spleen in the mothers were not different from those in the controls. In the young, no external and skeletal malformations were observed. Some abnormal changes in gestation such as abortion or resorption of fetuses were noted, but these observations were made in all groups, with an incidence of 21% in the L-glutamic acid hydrochloride group, of 25% in the monosodium L-glutamate group and of 27% in the controls. There were no external and skeletal malformations in the aborted fetuses (Yonetani, 1967).

10) Three groups of 6-10 female and 2 male rabbits were given "Glutamidine", a speciality pharmaceutical based on glutamic acid hydrochloride, in combination with or without vitamin B_6 in a period of pregnancy or pre-pregnancy for a month.

An oral daily dose of glutamic acid hydrochloride or vitamin B_6 was 25 mg/kg body weight. Hyperplasia of adrenals and endometrium, inhibition of spermatogenesis, generalized hyperemia, lowered reproduction rate, resorption of the fetus or abortion was observed in the parents treated with "Glutamidine". Hyperplasia of the adrenals, increased basophils, effects on the adenohypophysis, atrophy of the endometrium and testes, blockade of spermatogenesis, atrophy of fat tissue, muscular anomalies, atrophy of bone tissue, malformations of limb bones or poor growth was noted in the young (Tugrul, 1965).

In teratology studies using the chick embryo, adverse effects were noted in some reports (Landauer et al., 1952; Landauer, 1945 ; Aleksandrov et al., 1964), while other reports showed no obvious toxicity or teratogenicity (U.S. Food Protection Committee, 1970b). Although the chick embryo test may reveal a teratogenic potential of a food chemical, the results obtained may have but limited relevance to the evaluation of teratogenic hazard for man, not only because of species variation but also because of the likely differences in the concentration of the acutely admihistered and chronically ingested chemical at the critical stage and sites of embryogenesis (U.S. Food Protection Committee, 1970a).

In Tugrul's report, numerous adverse effects on both dam and offspring were recorded, but lack of experimental detail evaluation of this report difficult. The results of Yonetani's

experiments, which were essentially of the same design as Tugrul's, directly contradicted Tugrul's results.

In conclusion, no obvious adverse effects of monosodium L-glutamate (MSG) or L-glutamic acid on reproduction or teratogenic findings were noted in mice, rats and rabbits.

3-4. Mutagenicity and carcinogenicity

At present only the production of tumors in the intact animal is recognized as a valid test of carcinogenic activity (U.S. Food Protection Committee, 1970a). Both sexes of each of at least two species of animals should be used in the tests throughout their life span. In most cases these species would be rats and mice (FAO/WHO Joint Expert Committee of Food Additives, 1961). Assays for various mutagenicity tests, for example in microorganisms, have been proposed as short term tests for carcinogenicity (U.S. Food Protection Committee, 1970a).

1) One control group of 200 male mice and 2 groups of 100 male mice of C-57/Black strain received dietary levels of 0%, 1% or 4% of monosodium L-glutamate. There was no evidence of carcinogenicity possessed by monosodium L-glutamate at termination of the 2-year dietary feeding (Little, A.D. 1953b).

2) Groups of 35-61 male and 35-89 female rats of Sprague-Dawley strain received for 2 years dietary levels of 0%, 0.1% or 0.4% of monosodium L-glutamate. No significant differences

were noted on tumor incidence between the control and treatment groups (Little, A.D. 1953a).

3) Rats of the Sprague-Dawley strain were fed diets containing large concentrations of monosodium glutamate for extended time periods. The consequences of the regimen were explored in the vascular and reticuloendothelial systems. A marked increase of basket cells in the peripheral blood was noted after the experimental diet was instituted. After several weeks, large, abnormal lymphoid cells, similar to those characterized in the literature as "neoplastic cells", were observed. A third change associated with this diet was the presence of neutrophils which exhibited an abnormal nuclear pattern resembling the macropolycyte that Cooke described in some cases of granuloyctic leukemia. Notwithstanding these alterations, it is thought that the principal effect of monosodium glutamate upon the vascular system is not one of leukemogenesis, but rather one of endothelial injury with the transformation of endothelial cells into phagocytes which are unduly fragile and degenerate into basket cells (Greenberg, 1972).

4) Two experiments of the same procedure were carried out. 2 groups of male Charles River albino rats weighing between 250 and 300 g were treated with oral doses of either 0.2 g/kg or 5.7 g/kg of monosodium L-glutamate daily for 14 consecutive

days. Twenty-four hours after the last dose, each animal was inoculated with S. typhimurium, strain G46, a histidine auxotroph. After a 3 hour residence in the peritoneal cavity, the bacteria were recovered and the number that had mutated (reverted) to their protrophic form was determined. Dimethylnitrosoamine was administered to 4 male rats as a single intramuscular injection of 100 mg/kg to serve as a positive control. The number of bacteria recovered from monosodium L-glutamate treated animals was not increased over that of the control animals (spontaneous rate). The positive control animals had a 3 to 5 fold increase over the control rate. Monosodium L-glutamate was not mutagenic in this test system (Industrial Bio-Test Laboratories, 1973b).

5) Three groups of 12 Charles River albino mice were treated with a single oral dose, via gavage, of monosodium L-glutamate at levels of 0, 2.7 or 5.4 g/kg body weight. Effects on male germinal cells were monitored by mating treated animals with groups of 3 untreated females for each of 6 consecutive weeks. Females were sacrificed at mid-term of pregnancy. The uterus was exposed and carefully examined for signs of early embryonic death, which are observed to be deciduomata. Animals in the 2.7 g level had slightly lower mating indices than control animals. However, mating indices in the 5.4 g level were comparable to those of controls. The lowered mating indices in the 2.7 g level were not considered to be of biological significance. When sacrificed, females

that had mated with treated males from either group had numbers of implantations, resorptions, and embryos similar to those of females that had mated with control males. Mutation rates, calculated from these values, for treated animals compared favorably with those for control animals (Industrial Bio- Test Laboratories, 1973a).

In conclusion, there is no evidence, from the tumor incidence in mice and rats through their life span, that monosodium L-glutamate is carcinogenic. A dominant lethal assay in mice and a host mediated assay in rats showed no mutagenic activity of monosodium L-glutamate.

3-5. Effects on retina

In 1957, Lucas et al. reported a retinal lesion induced by successive subcutaneous injections of MSG in suckling mice. After that, several studies relating to the MSG-induced retinal lesion were performed by light and electron microscopy and electroretinophysiology in mice, rats, rabbits and monkeys.

1) Seventy suckling mice, Strong A2 (Glaxo) strain, were subcutaneously injected with MSG once a day from the 2nd to 16th day of age following the increasing dosage schedule from 2.20 to 5.40 g/kg body weight. Survivors (39) were examined at ages of between 2 and 40 days and retinal lesion was found from all of them. Necrotic changes affected the ganglion cells, the inner fiber layer, and some of the bipolar cells within a few hours of injection, while the visual-cell layer was not affected. In very young animals, the damage to the inner layers was more extensive and groups of rod cells protruded through the outer limitting membrane. Six pregnant mice were subcutaneously treated with 40 mg (1.0 to 1.5 g/kg b.w.) MSG/day, throughout gestation and/or lactation and 40 sucklings were examined at birth or 17 days later. None of them showed retinal lesions. In adult mice, single doses of 4 to 8 g/kg body weight produced only a partial retinal lesion (Lucas et al., 1957).

2) Six litters of Swiss albino mice (35 animals) and 4 litters of C3H/HeJ mice (10 animals) were given daily subcutaneous injection of MSG for 18 days or 10 days beginning on the second postnatal day according to the increasing dosage schedule from 2.2 to 5.8 g/kg body weight. The light and electron microscopic studies were performed at intervals of two months and one year after treatment. At two months, eyes were noted smaller than that of the control. The lenses averaged 65% of the weight of those of the control and the neuronal cells of the inner layer had been reduced from 5-7 nuclei deep to about 1-2 (Cohen, 1967).

3) A pattern of degeneration affecting certain cells of the inner retina was induced in suckling mice by a single subcutaneous injection of MSG at a dose level of 4 g/kg body weight. Lesion severity was related to developmental age. The maximal lesion was induced by a single injection in the 10th and 11th postnatal days. Similar lesions resulted from daily treatments on the 1st to 10th day inclusive. Retinas were studied by electron microscopy at serial post treatment intervals. Swelling of dendrites and cell bodies occurred as early changes in all neurons affected. Several patterns of degeneration leading to neuronal necrosis and some reactive changes in several non-neuronal cell types were observed. Phagocytosis was observed in the post treatment period and nearly all products

of degeneration were removed from the retina within 48 hours of treatment (Olney, 1969b).

4) MSG was given intraperitoneally from day one to two weeks of age by an increasing dosage schedule of 2.20 to 5.40 g/kg. Test animals were 31 litters of Sprague-Dawley rats. The retinas and optic-nerves and -tracts were examined by electron microscopy after two to ten months. The nerve cells in the ganglion cell layer and in the inner nuclear layer were destroyed with a few exceptions. The rest of the cells in the inner part of the retinas consisted of Mullerian neuroglial cells, astrocytes, occasionally observed oligodendrocytes and scattered glial cells of intermediate type. The photoecepter cells and the epithelial cells were relatively unchanged (Hansson, 1970b). Essentially similar results were observed by scanning electron microscopy (Hansson, 1970a).

5) Newborn mice were injected intraperitoneally with a single daily dose of MSG from the 2nd to 18th neonatal day according to the increasing schedule of 2.2 to 5.8 g/kg body weight. Permanent alterations in the b-wave of the electro-retinogram and inhibition of formation of the inner retinal layers were observed in those animals treated for more than seven days. Animals treated for less than seven days showed various degrees of retardation of retinal development, while blockage of b-wave was reversible (Potts et al., 1960).

6) A study was performed on the retina of adult albino rabbits treated with MSG to evaluate ERG, histology and histochemistry. Twenty one adult rabbits were divided into five groups and injected intraperitoneally once a day for 16 consecutive days with 2.0, 1.0, 0.5, 0.25 and 0.1 g MSG/kg body weight, respectively. With 2.0 g/kg MSG treatment, relatively intense degeneration was produced on the total layers of the retina and the amplitudes of a and b-waves simultaneously decreased to smaller than one half of pre-treatment response. The minimum dose to produce the degeneration was 0.25 g/kg (Hamatsu, 1964).

7) Nine adult albino rabbits were intraperitoneally given successive injection of MSG 0.1 g/kg body weight and killed on 10th, 30th and 50th days of treatment. Histological changes were observed in the inner segment of the receptor cells and the outer plexiform layer. The relation between number and size of synaptic vesicles and the results of electrophysiological studies were discussed (Kobayashi, 1970).

8) On the feeding study in squirrel monkeys, no abnormality was found by the electroretinogram. Neither dendritic swelling in the inner plexiform layers nor necrosis in the ganglion cells was found in the ultrastructual examination of the retina (Wen et al., 1974).

3-6 Effects on brain

1) Mice

In 1969, acute brain lesions, such as intracellar edema and neuronal necrosis in the preoptic and arcuate nuclei of the hypothalamus were reported in neonatal mice that received subcutaneous injections of monosodium L-glutamate (MSG) at doses from 0.5-4 g/kg body weight (Olney, 1969a). To date, a number of related experiments have been carried out in mice, rats, rabbits, chicks, dogs and monkeys by various researchers. The neuropathological character of the lesions, dose-lesion relationship, physiological and biochemical changes were made clear in mice in comparison with the other animal species examined. The experimental methods and results of the studies in mice are summarized briefly in the Table 4.

As shown in Table 4, there are many studies which try to confirm the possibility that the administration of MSG under various conditions may produce lesions in the brain of infant or adult mice.

The early reports (Olney, 1969a, 1969b, 1970) regarding the susceptibility of the mouse retina and arcuate nucleus of the hypothalamus to neuronal damages after subcutaneous administration of MSG are not supported by the following studies. The light microscopic examination of the brains of rats by Olney's methods failed to reveal any effect of single subcutaneous injection upon the lateral preoptic nucleus,

Table 4. Experimental methods and results of studies on brain lesion in mice treated with MSG

Route	Dose g/kg body wt. (No. of dose)	Age (day)	Treatment-sacrifice interval (h)	Brain lesion		Reference
				Site	Nature	
s.c.	0.5-4.0 (single)	2-9	1-48	Hypothalamus	Neuronal necrosis	Olney, 1969
	5-7 (single)	Adult				
	2-4 (single)	3-10	3-72	Hypothalamus	Microglial necrosis	Arees et al., 1970
	6-10 (single)	Adult				
	3.4 (single)	10	15(min.)-8	Hypothalamus	Neuronal necrosis swelling of microglial and ependymal cells	Olney, 1971
	2-4 (daily)	1-10		Arcuate nuclei	Absence of neurons	
	1 (single)	3, 12	24	———	———	Oser et al., 1971 1973
	1 (single)	2-4	3, 6	Hypothalamus	Neuronal necrosis	Murakami et al., 1971
	5 (single)	Adult	3	Hypothalamus	Neuronal necrosis	

Table 4. (continued)

Route	Dose g/kg body wt. (No. of dose)	Age (day)	Treatment-sacrifice interval (h)	Brain lesion		Reference
				site	Nature	
s.c.	1, 4 (single)	5-7	1-24	Hypothalamus	Neuronal necrosis	Abraham et al., 1971
	0.1-4 (single)	1-2	1-48	Hypothalamus	Neuronal necrosis ($\geqq$1.0g/kg)	Matsuyama et al., 1970 1973
	4 (single)	0	4	Hypothalamus	Neuronal necrosis	Nagasawa et al., 1974
p.o.	0.25-2.00 (single)	10-12	5	Hypothalamus	Neuronal necrosis ($\geqq$0.5g/kg)	Olney, et al., 1970
	2 (single)	9-10	no information		Brain lesion	Geil et al., 1970
	1 (single)	3, 12	24	——	——	Oser et al., 1971 1973
	1, 4 (single)	5-7	1-24	Hypothalamus	Minimal neuronal damage	Abraham et al., 1971
	1 (single)	10	5	Hypothalamus	Neuronal necrosis	Burde et al., 1971
	4 (single)	neonate	20(min.) - 24	Many regions adjacent to cerebrospinal fluid	Neuronal recrosis	Lemkey-Johnston et al., 1972

Table 4. (continued)

Route	Dose g/kg body wt. (No. of dose)	Age (day)	Treatment-sacrifice interval (h)	Brain lesion		Reference
				Site	Nature	
p.o.	2-9 (daily)	6-10	11 (days)	——	——	Wen et al., 1973
	1-4 (single)	7-10	15 (min.) - 48	Many regions adjacent to cerebrospinal fluid	Neuronal necrosis	Lemkey-Johnston et al., 1974
	0.25-4 (single)	5-9	10 (min.) - 48	Many regions adjacent to cerebrospinal fluid	Neuronal necrosis	Reynolds et al., 1974
p.o. dietary feeding	2-8	fetus-adult	——	——	——	Yonetani et al., 1970
i.p.	6-10 (single)	adult	3-72	Hypothalamus	Microglial necrosis	Arees et al., 1970
Maternal (s.c. in dam)	5 (single)	17, 18 (in utero)	3, 6, 24	Hypothalamus Hippocampus	Neuronal necrosis	Murakami, 1971
			1 week after birth			
			3 weeks after birth	——	——	

arcuate nucleus and median eminence (Adamo et al., 1970), and the examination of the eyes and of the preoptic and arcuate nuclei of the hypothalamus revealed no dose-related histomorphological effects in infant mice sacrificed at 24 hours after a single subcutaneous or oral administration (oser et al., 1971).

The observation that the arcuate nucleus of the hypothalamus was primarily affected by MSG administration, was questioned by Arees et al. (1970). They claimed that although they confirmed the existence of a necrotic lesion in mouse brain, the lesion was identified to be very specific for glial cells and apparently did not involve neurons within the arcuate region. Neuronal damage was confirmed (Abraham et al., 1971) by the ultrastructural study on the hypothalamus in mice, in which the authors found arcuate neuron damage following MSG administration. The action of MSG was directed against both neuronal and glial cells in subcutaneously treated mice, while a predominantly glial reaction was found in orally treated mice.

Critical aspects of the route of administration, developmental ages and post-treatment intervals therefore became clearer.

On the critical aspects of the route or the type of administration, most studies manifested hypothalamic damage in infant and adult (only in Olney, 1969., Arees et al., 1970), following subcutaneous injection, and in infant animals with oral administration.

Tissue changes in the brain such as neurolysis and neurophagia were observed in most of the test and control animals receiving MSG by subcutaneous or oral administration thus suggesting lack of a dose related specific histopathologic effect (Oser et al., 1971).

Mice which survived the 5-day treatment period at dosage of 2-9 g/kg of MSG p.o., were found to have no evidence of damage in the hypothalamic region (Wen et al., 1973).

No brain lesion was observed in mice following the dietary administration of 2-8 g/kg of MSG, throughout the prenatal (through mother), postnatal and adult periods (Yonetani et al., 1970).

Many critical studies on the susceptibility of various ages were performed in mice, but brain damage persisted at any stage of infant and adult mice. The minimal dosage of MSG to produce neuronal lesions was reported to be more than 0.5 g/kg with subcutaneous injection (Olney, 1969) or per oral administration (Olney, 1970).

By using Olney's method, the threshold dosage was affirmed to be between 1 and 2 g/kg for mice given various doses of MSG subcutaneously (Matsuyama et al., 1973).

The effect of the period between administration and sacrifice was investigated. Development of lesions was observed in mice which were treated subcutaneously on the 10th postnatal day with a 3.4 g/kg dose of MSG in 10 % aqueous solution. The sacrifice was at intervals from 15 minutes to 8 days following the treatment. In the study,

an initial stage of acute intracellular edema, detectable by light microscopy within the first hour, reached peak severity at approximately 6 to 8 hours following MSG by either the subcutaneous or oral route of administration. In the infant hypothalamus, susceptible nerve cells undergo rapid necrosis, and are phagocytized, degraded, and evacuated from affected area within 24 to 48 hours after MSG treatment (Olney, 1971).

A series of mice were given MSG (4 g/kg) in a 20 % solution via stomach tube and killed at 10, 15, 20 and 30 minutes, and 1, 1.5, 3.6, 12, 24, 36 and 48 hours after MSG treatment. Edematous astrocytic somatous brain lesions were observable at 15 minutes by electron microscopy and at 20 minutes by light microscopy. For the first three hours after MSG administration, astrocytic and neuronal edema, vacuolation of neurons and nuclear pyknosis were observed.

Between three and six hours after treatment of MSG, phagocytosis began. At six hours, the number of pyknotic nuclei has greatly increased. At twenty-four hours, the appearance of the lesion by light microscopy had changed markedly (Lemkey-Johnston, 1974).

Similar acute hypothalamic lesions were reported in neonatal mice by subcutaneous injection of 3 g/kg of monosodium L-aspartate, L-glutamic acid and L-cysteine (Olney et al., 1970).

No lesions were reported in mice administered approximately the same amount of sodium chloride as MSG (Olney et al.,

1970; Olney, 1971; Burde, 1971), but acute brain lesions which were different in the sites and natures from those of MSG administration were reported in neonatal mice orally given sodium chloride equimolar to 4 g/ or 8 g/kg of MSG (Reynolds et al., 1974).

There are some experimental results showing effects of MSG administration on the reproductive function: sterility (Olney, 1969) or hormonal disturbance (Nagasawa et al., 1974) was observed in mice injected with MSG subcutaneously at their neonatal stage. On the other hand, other reports indicated no remarkable change in the reproductive system or in the sexual cycle of female mice treated with MSG under essentially the same conditions mentioned above (Matsuyama et al., 1970, 1973). There is no obvious evidence that the dietary MSG influenced the reproductive function (Yonetani et al., 1970).

Two reports indicated that mice grew up to be obese by subcutaneous injection of approximately 3 g/kg body weight or more of MSG at their neonatal stage(Olney, 1969; Matsuyama et al., 1970). However, no obese animals were observed when MSG was given either orally for an extended period of time or subcutaneously (not more than 0.5 g/kg body weight) from or at the neonatal stage (Wen et al., 1973; Yonetani et al., 1970; Matsuyama et al., 1973).

2) Rats

Acute brain lesions the same as neonatal mice treated with MSG were reported in neonatal rats administered MSG subcutaneously (approximately 2 g/kg body weight or more) or orally (not less than 4 g/kg body weight) (Burde et al., 1971; Everly, 1971), while no acute lesions were observed in other experiments under the similar conditions (Adamo et al., 1970; Matsuyama et al., 1970, 1973; Oser et al., 1971).

There is no evidence that weanling rats administered MSG at dietary levels of 16.7 % or 28.6 % for 5 weeks showed the brain lesions (Wen et al., 1973). No brain lesions were histopathologically observed in rats treated with MSG by gastric tube, at daily doses of 5 g/kg body weight, from immediately after weaning, for 220 days (Shimizu et al., 1970).

A report suggested that rats consecutively injected with MSG for an extended period of time, beginning on the 2nd day of age, might result in changes in the areas of the hypothalamus responsible for elaboration of the hypothalamic releasing hormone. In the rats after 40 days of the treatment, the weights of the adrenal and anterior pituitary glands as well as the content of growth and luteinizing hormones in the anterior pituitaries were significantly reduced (Redding et al., 1971).

In another report, no abnormalities were noted in time of sexual maturity, estrous cycle and fertility in the rats treated with MSG by intragastric tube,at daily doses of 5 g/kg body weight, from immediately after weaning, for 220 days (Suzuki et al., 1970).

3) Guinea-pigs

Three-day-old guinea-pigs were given MSG 1 g/kg subcutaneously. Numerous necrotic neurons in the region of junction of median eminence and arcuate-periventricular nucleus of the hypothalamus were observed light-microscopically after five hours treatment. This result suggested that the guinea-pig was susceptible to glutamate-induced brain damage (Olney et al., 1973).

4) Rabbits

Microscopic examination was performed on the brain and pituitary from 10 rabbit pups (5 males and 5 females) removed from does which had been fed MSG at a level of 8.25 % for two to three weeks prior to mating and during the subsequent 29 to 30 days prior to Caesarean section. Evidence of neuronal necrosis or other pathologic alteration was not apparent in the sections examined. Similar investigations on 10 rabbit pups (5 males and 5 females) from the does receiving MSG at the levels of 0.1 and 0.825 % revealed neither apparent neuronal necrosis nor other pathologic alteration in the sections (Hazleton, 1966).

5) Dogs

Twenty groups of 3 dogs at 3 days or 35 days of age received subcutaneously or orally a single dose(1 g/kg) of monosodium glutamate, monopotassium glutamate, sodium chloride, sodium gluconate or water and were killed at 24 hours after

treatment. Light microscopic examination of the hypothalamus revealed no significant differences among all the test and control groups (Oser et al., 1971).

6) Monkeys

One premature male infant rhesus monkey was subcutaneously injected with a single dose of MSG 2.7 g/kg body weight at 8 hours after birth. The infant showed no manifestations of a central nervous system disturbance and was killed at 3 hours after treatment for light and electron microscopy. A lesion affecting the periventricular-arcuate region of the hypothalamus was observed by light microscopy. Electron microscopy revealed that the dendrites and cell bodies of neurons were primarily affected (Olney et al., 1969).

Four, 4-day-old rhesus monkeys were treated with 4 g/kg body weight MSG: Two were given orally by stomach tube and two received by subcutaneous injection. Two controls were given equivalent amount of water orally by tube and subcutaneously. The animals were killed at 3 or 24 hours after the treatment. No lesions were observed in the lateral preoptic nuclei, arcuate nuclei and median eminence. The authors suggested that the varied effects of MSG on mice and monkeys were attributable to the permeability of the blood-brain barrier, consequent upon the disparate degrees of myelination of the central nervous system attained at birth.(Abraham et al., 1971).

Sixteen infant monkeys (M. irus and M. mulatta) were

orally given single doses of MSG (1, 2 and 4 g/kg body weight) by stomach tube, and five controls received only water. No significant morphological differences could be detected by the light and electron microscopy in the periventricular-arcuate area between the controls and MSG-treated animals. In both control and treated infants, poorly fixed tissue appeared similar, at the ultrastructural level, to that described in a newborn monkey after MSG treatment (Olney et al., 1971). The authors suggested that the central nervous system of the newborn primate and the newborn rodent were hardly comparable with respect to both functional and morphological indices of maturation (Reynolds et al., 1971).

Four infant rhesus monkeys were subcutaneously injected with 2.7, 2.7 and 4.0 g/kg of MSG and with 1.2 g/kg body weight of NaCl, respectively. Five infant monkeys were orally given 1.0, 2.0 and 4.0 g/kg of MSG and 0.3 and 0.6 g/kg of NaCl, respectively. Oral treatments were prepared by adding enough NaCl or MSG to a 50/50 mixture of water and skim milk in a volume of 20 ml/kg body weight. Hypothalamic lesions identical to those of rodents were identified by light microscopy and verified with the electron microscope in each infant monkey given MSG. No cellular pathology was detected in the hypothalami of NaCl-treated controls. MSG at 1 or 2 g/kg produced small focal lesions confined primarily to the restro-ventral aspect of the infundibular nucleus. High subcutaneous doses caused lesions which spread throughout and sometimes beyond the

infundibular nucleus. In all cases, rapid necrosis of neurons (within 5 h) was a striking feature of the MSG-induced reaction pattern. From blood glutamate levels the authors suggested that the threshold for lesion formation in 1-week-old rhesus monkeys might be in the range of 20 mg% (Olney et al., 1972).

Six monkey fetuses (Macaca species) received approx. 4 g/kg monosodium glutamate via umbilical vein-infusion. The fetuses were removed by Ceasarean section after 2-6 hr post-treatment. Brain taken from the fetus was processed for microscopic examination. The hypothalamic areas of the brain were found to be normal in all fetuses. No pyknotic nuclei, tissue edema, neuronal loss or other evidence of cellular necrosis were observed in the arcuate region (Reynolds et al., 1973).

Ten newborn male and female squirrel monkeys were fed diet containing 0, 4.8, 9.1 or 16.7 % MSG for 9 weeks and the retina and hypothalamus evaluated histopathologically during the 11th week. Electron microscopy of the hypothalamus and retina revealed no differences between groups. No pyknotic nuclei, lysosomal bodies, phagocytic vacuoles or other evidence of cellular necrosis were observed in the hypothalamus of any animal (Wen et al., 1973).

Fourteen neonatal rhesus monkeys of various ages ranging from 2-99 days were orally administered a single dose of 2 g/kg body weight of MSG as a 20 % aqueous solution by intragastric tube. Seven monkeys of respective ages served as undosed

controls. Two rhesus monkeys 80 days of age were orally administered 4 g/kg of MSG one served as an undosed control. All animals were sacrificed approximately four hours after the treatment. Six pregnant rhesus monkeys received daily 4 g/kg body weight of MSG, via gavage, during the last one-third of pregnancy. Four pregnant monkeys served as undosed controls. All animals were killed four hours after birth. Examination was made by light and electron microscopy for evidence of changes in the hypothalamic region. There was no evidence in any instance of any change that could be attributed to MSG, although there were artifacts in some inadequately fixed areas from both test and control animals (Newman et al., 1973).

Thirty-two neonatal cynomolgus monkeys of 3-4 days of age were administered, in a single oral or subcutaneous dose, monosodium L-glutamate (MSG), monopotassium L-glutamate, sodium chloride, sodium gluconate or distilled water. Assignment of animals treated with MSG and water is as follows:

Dosage (g/kg b.w.)	Route of Administration	No. of Animals Treatment-Sacrifice Interval 3h	6h	24h
1 g MSG (10% solution)	oral	3	1	2
	s.c.	1	-	1
4 g MSG (20% solution)	oral	2	1	1
4 g MSG (with baby food)	oral	1	-	-
Water	oral	2	-	2
	s.c.	1	-	-

No changes that could be attributed to MSG, or the other test substances, were observed in the hypothalamus or eye using detailed microscopic examination of all animals. (Oser et al., 1971, 1973).

3-7 Effects of massive ingestion in human

In 1968, "Chinese Restaurant Syndrome (CRS)" was first reported by Kwok who had experienced a syndrome after eating Chinese food. This syndrome, which began 15 to 20 minutes after eating the first dish, lasted for about two hours without any lasting effects. The most prominent symptoms were numbness at the back of the neck, general weakness and palpitation (Kwok, 1968). Several similar communications followed (Schaumburg, 1968 ; McCaghren, 1968 ; Menken, 1968 ; Beron, 1968 ; Kandall, 1968 ; Gordon, 1968).

Ambos et al., (1968) and Schaumburg et al., (1968) claimed that monosodium L-glutamate (MSG) caused CRS like symptoms.

Pharmacological studies were carried out in human subjects given MSG orally or intravenously. In appropriate doses , MSG caused burning sensations in the face and trunk, facial pressure and chest pain. The oral threshold range for minimum symptoms in 36 subjects was 1.5 to 12 g. The intravenous threshold range in 13 subjects was 25 to 125 mg. There was no apparent relation between threshold and body weight, sex or age. The subjects who showed higher oral threshold did not always show higher intravenous threshold values. In intravenous administration, burning sensation appeared at first within 20 seconds, next chest pain, and finally facial pressure. In oral administration, subjects experienced only one or two of the three symptoms

after 15 to 25 minutes, Dose-related response of the symptoms were observed both in the oral and intravenous administration. Similar effects were obtained by L-glutamic acid (5 g), DL-glutamic acid (5 g) and monopotassium L-glutamate (4 g), but no effects were noted with monosodium D-glutamate (7 g), monosodium L-aspartate (5 g), sodium chloride (10 g) and glycine (5 g) (Schaumburg et al., 1969).

An epidemiological survery was made with 912 individuals to determine if they experienced any symptoms after the intake of a prepared Oriental type noodle soup containing 0.61-1.36 g MSG per serving. No characteristic symptoms described as CRS were induced in any of the individuals examined by intake of the noodle containing MSG. MSG has never caused any special symptom under the circumstances of normal dietary intake in Japan (Ichimura et al., 1970a).

Clinical experiments on the effects of MSG were studied by a double blind method, using capsules containing 2.2, 4.4, and 8.7 g of MSG respectively and placebo of capsules containing lactose, on a total of 61 healthy male volunteers. The intake was either 30 minutes after lunch or 15 hours after dinner. In the experiments 30 minutes after lunch, no significant differences in incidence of the symptoms were noted between the placebo and MSG treated groups. Similarly 15 hours after dinner,

the frequencies of CRS like symptoms did not show any significant difference among groups given 2.2 or 4.4 g of MSG and placebo. Following the highest level of MSG, only three cases (6%) experienced burning sensation and/or pressure feeling, but no subject experienced all three symptoms simultaneously. The objective effects including blood pressure, pulse rate, electrocardiogram (EKG), and sodium and glutamic acid levels in blood were measured after administration of MSG in 5 persons who had experienced no symptoms, and 9 complained of some symptoms. There were no changes in blood pressure, pulse rate, EKG, or sodium content of the blood in both groups. No significant differences between both groups were observed in increase in plasma glutamic acid (Ichimura et al., 1970b).

Up to date, many clinical studies on CRS have been reported.

Twenty-four healthy volunteers (17 males and 7 females) from 18 to 34 years of age were given 150 ml of beef broth with or without 3 g MSG followed other dishes at lunch time, using double blind and crossover techniques. Subjective symptoms and objective parameters such as arterial blood pressure, pulse and respiration rate were recorded every 20 minutes during the following 3 hours. No differences were observed in their subjective symptoms or objective measurements between MSG treatment and control groups

(Morselli et al, 1970). Further studies were reported by them. MSG was administered at lunch time to 73 healthy subjects (38 males and 35 females) 17-76 years of age, using a double blind design with each subject acting as his own control. Care was taken to balance the sexes, and to avoid any psychological effect of grouping. Both subjective and objective evaluations were carried out 20, 40, 60, 90 and 120 minutes after drinking bouillon with or without 3 g MSG. No significant differences between the control and MSG groups were observed in the incidence and nature of subjective symptoms such as headache, flushing, facial pressure, chest pressure, gastric distress, nausea, perspiration, prostration or any other. With regard to the objective parameters such as arterial blood pressure and the pulse rate, no clinically meaningful differences were observed between the two groups. No remarkable differences were observed between males and females (Zanda et al., 1973).

Ninety-eight male volunteers of varying age received a single 5 g dose of MSG dissolved in either tap water or diluted chick soup stock. Each volunteer filled out a questionnaire consisting of 14 symptoms. Approximately one-half of the subjects affirmed one or more symptoms, but only one of these appeared to have "reacted" to MSG, (Rosenblum et al., 1970). More detail experiments were reported by the same outhers. Single and double blind

studies were done with single oral doses of MSG in 99 male volunteers from 21 to 59 years of age on a fasting stomach (18 hours after last meal). Ninety-eight received 5 g of MSG in tap water or in chicken stock in single blind studies. The controls were given chicken stock alone or containing NaCl equimolar to MSG. A total of 169 doses including controls were given. Based on the questionnaire, the incidence of complaints in the treated and control groups ranged from 33-80% and 20-29%, respectively. There was a low incidence of most complaints except for light headedness and tightness in the face. Six received 8 g and 5 received 12 g of MSG in chicken stock in double blind studies. In these studies, no significant differences between MSG treatment and NaCl treatment groups were observed in blood pressure, radial pulse and clinical chemistry such as serum calcium, phosphorus, glucose, urea nitrogen, uric acid, total cholesterol, total protein, albumin, total bilirubin, alkaline phosphatase, LDH [E.C. 1.1.1.2.7.] and GOT [E.C. 2.6.1.1.]. No subject reported or was observed to have experienced the triad of symptoms described as the CRS (Rosenblum et al., 1971).

Fourteen subjects were fed 25-147 g MSG per day for 14-42 days along with a chemically defined diet or a typical diet. All subjects tolerated the regimen well and showed no changes in neurologic or hepatic function, body weight, irritability, appetite or mentation. None developed the CRS.

The only biochemically demonstrable effect of MSG was a decrease in serum cholesterol and associated β-lipoproteins (Bazzano et al., 1970).

Fourteen healthy volunteers (10 males, 4 females) were given orally 150 mg/kg body weight of MSG in 150 ml water after an overnight fast. During the tests, the subjects were observed closely and their complaints were recorded. Objective evaluations such as pulse rate, pupil size, facial color, perspiration, blood pressure and blood cholinesterase (ChE) were observed. All subjects developed definite signs and symptoms of the MSG loading. No abnormal changes in blood pressure or pulse rate were noted during the 3-hour observation period after a loading of MSG. EKGs were normal during the first one hour. A decrease of about 30% in the plasma ChE activity was observed at 60 minutes after MSG ingestion. In other experiments, complaints caused by MSG loading were reduced by atropine treatments, and were potentiated by prostigmine treatments. The authors suggested that CRS was a transient acetylcholinosis (Ghadimi et al., 1971).

Six lactating women received a 6 g load of MSG in conjunction with water or 240 ml of Carnation Slender after an overnight fast. None of the subjects reported symptoms associated with CRS at this dose (Stegink et al., 1972).

Human susceptibility to MSG was studied on 77 normal volunteers (44 males, 33 females) by a single placebo-controlled exposure. The subjects received 150 ml tomato juice containing 5 g of MSG or placebo (juice alone). Twenty-five persons (32%) reported one or more symptoms after MSG intake, and 11 persons reported one symptom after placebo intake. There was no report of the triad of symptoms which characterize the so called CRS. There was a significant difference between the frequency of occurrence of symptoms in males (25%) and females (42%). Neither resting level of blood glutamate nor the level achieved after MSG ingestion served to predict reaction or non-reaction in a subject. Double blind experiments were carried out in 22 of the 25 subjects reporting symptoms due to MSG ingestion. When the subjects were given 150 ml solutions containing 1, 2, 3, 4 and 5 g or MSG or placebo, percentage of positive responses were 6.8, 20.5, 56.4, 75.6, 80.4 and 11.1-20.6, respectively (Kenny et al., 1972). Effect of concurrent caloric intake on the response to oral MSG was studied in susceptible subjects. Thirteen subjects were selected from the reactor group in the studies mentioned above. Subjects ingested lunch-type snacks containing 150 ml tomato juice with or without 5 g of MSG. Four snacks were used. The compositions of the snacks were selected to provide 400-470 calories with, in one case, a high fat low protein mixture, in another a high

carbohydrate low protein mixture and in the two other cases high protein content with differing contents of natural L-glutamic acid. Responses recorded following the ingestion of either placebo or the juice containing MSG differed in no qualitative way from those reported in the previous study. The latency of symptom onset in series was consistently longer than when MSG was administered in juice unaccompanied by snack. The response rate to snacks containing 5 g of added MSG was significantly lower in cases of high carbohydrate low protein mixture, and high protein mixtures than in the case of high fat low protein mixture or the previously determined response to this dose of MSG given without a snack. A snack with the juice halved the frequency of symptom occurrence at the 5 g level of MSG. Natural L-glutamic acid in the form of protein produced no additional response in this susceptible subject (Kenney, 1974).

There are many reports on therapeutic applications of L-glutamate. These included long-term treatment, in some cases over a number of years, with high dosage of glutamic acid or MSG. The daily doses were generally in ranges of 5-20 g. Many investigators observed nausea or vomiting as side effects during the administration of high doses.

Twenty-three adult subjects were given daily doses of 12-36 g L-glutamic acid (as sodium salt) during 4 months. In two subjects vomiting occurred on several occasions after L-glutamate administration. Many complaints of a feeling of nausea, particulary in the morning, were reported. No other effect was noted throughout the whole period of the experiment (Milliken et al., 1951).
A total of 101 hospitalized children, ranging in age from 2 weeks to 13 years, were administered 1.75 g/kg /day of L-glutamic acid over periods of 6-24 months. There was no effect on the physical growth and development, liver, kidneys or hematopoietic system. Nausea and vomiting occurred in 13 children, but in many cases these symptoms disappeared during the course of the treatment. Headaches occurred in only a few instances (Jäger-Lee et al., 1954). Thirty patients were given 15 g L-glutamic acid (as sodium salt) per day in 3 separate doses over 11 months. No effect on basal metabolic rate, EEG, EKG, pupillary diameter, blood pressure, heart rate, respiration rate, oral temperature and body weight was noted. Following the administration of a daily dose of 45 g L-glutamic acid in 3 separate doses, a few subjects reacted by vomiting (Himwich et al., 1955).

The relationship between plasma glutamic acid level and the occurrence of nausea and vomiting were studied by intravenous administration of glutamic acid solutions in human subjects. When the serum free glutamic acid level

reached 12-15 mg/100ml, nausea and vomiting occurred in more than half of the subjects (Levey et al., 1949). These side effects were not specific to glutamic acid: aspartic acid also caused the same symptoms (Smyth et al., 1947).

In conclusion, a massive ingestion of monosodium L-glutamate in susceptible persons has been suggested to cause certain kinds of symptoms, so-called "CRS". The similar symptoms, however, were also observed in volunteers who received several kinds of controls. All the symptoms characteristic of CRS are subjective and transient. It is difficult, if not impossible to find objective symptoms. Therefore, these facts indicate that application of the double blind study should be essential for the elucidation of CRS. Several experiments mainly by the double blind method have been reported (Ichimura et al., 1970b; Morselli et al., 1970 ; Rosenblum et al., 1971 ; Kenney et al., 1972 ; Zanda et al., 1973 ; Kenney, 1974). These studies show that there are no significant differences in the incidence of symptoms between control and treated groups within a 3 g to 4.4 g dose of monosodium L-glutamate in a general unselected population.

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